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Inhibition of Noroviruses by Piperazine Derivatives

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Abstract

There is currently an unmet need for the development of small-molecule therapeutics for norovirus infection. The piperazine scaffold, a privileged structure embodied in many pharmacological agents, was used to synthesize an array of structurally-diverse derivatives which were screened for anti-norovirus activity in a cell-based replicon system. The studies described herein demonstrate for the first time that functionalized piperazine derivatives possess anti-norovirus activity. Furthermore, these studies have led to the identification of two promising compounds (**6a**, **9l**) that can be used as a launching pad for the optimization of potency, cytotoxicity, and drug-like characteristics.

Keywords

norovirus; inhibition; piperazine derivatives

Noroviruses belong to the *Norovirus* genus of the *Caliciviridae* family and are the most common cause of acute viral gastroenteritis in the U.S. and worldwide.^{1–3} Noroviruses are highly contagious and outbreaks in cruise ships, schools, hospitals and nursing homes, are associated with significant morbidity and mortality. Although infection by noroviruses is generally self-limiting, the disease constitutes an important health problem and a potential bioterrorism threat because of its highly contagious nature and morbidity. The problem is further compounded by a dearth of small-molecule therapeutics or vaccines. Indeed, only a limited number of studies aimed at the discovery of therapeutics for norovirus infection have been reported in the literature.^{4–6} We have recently described the inhibition of noroviruses by cyclosulfamide derivatives and have used a scaffold hopping strategy to identify additional series of compounds with anti-norovirus activity.^{7–9} During the course of those studies, a cyclosulfamide-based piperazine hit was identified that exhibited noteworthy anti-norovirus activity. The piperazine scaffold is a privileged structure^{10–12} capable of binding to multiple receptors with high affinity. It is a recurring structural motif in a large number of biologically active molecules.¹³ Based on the foregoing, we hypothesized that functionalized piperazine derivatives may exhibit anti-norovirus activity. To explore this hypothesis, small, focused libraries of piperazine derivatives were synthesized and screened for anti-norovirus

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activity using a replicon-based system. We describe herein the results of synthetic and biochemical studies related to the discovery of piperazine derivatives (structure (I), Figure 1) as anti-norovirus agents.

A series of structurally-diverse piperazine derivatives was synthesized in order to develop preliminary structure-activity relationship studies and to identify a hit suitable for use in a hit-to-lead optimization campaign.^{14–15} The anti-norovirus effects of the synthesized compounds¹⁶ were examined in NV replicon-harboring cells (HG23 cells)^{17–20} and the results are summarized in Table 1.

Benzyl piperazine was initially coupled to a series of carboxylic acids to generate compounds **1a-i** (Scheme 1) which were subsequently screened in a cell-based replicon system. A few of the compounds had low μM anti-norovirus activity (compounds **1a-1b** and **1f**, Table 1), with compound **1f** having a better therapeutic index than the other two compounds. Furthermore, anti-norovirus activity was found to be very sensitive to the nature of the ring substituent. These observations provided preliminary support of the hypothesis that suitably-functionalized piperazine derivatives possess anti-norovirus activity.

A series of substituted 1*H*-1,2,3-triazole-4-carboxylic acids **2a-e** were then prepared using click chemistry methodology^{21–23} from propargylic acid and the corresponding azides. Subsequent coupling to 1-benzyl piperazine dihydrobromide gave compounds **3a-e** (Scheme 1) which were found to be inactive.

The triazole ring was then replaced by γ -lactam ring. Thus, compounds **4a-d** were constructed using dimethyl itaconate and the corresponding primary amines²⁴. Subsequent hydrolysis of **4a-d** with 10% potassium hydroxide gave compounds **5a-d** which were coupled to 1-benzyl piperazine to give compounds **6a-d** (Scheme 2) of which the (*p*-methoxyphenyl) substituted derivative **6a** had a therapeutic index of ~22. Thus, the replacement of the triazole ring by a γ -lactam ring was highly beneficial.

To further probe how the nature of the substituents attached to the piperazine scaffold affects anti-norovirus activity, a series of (*m*-phenoxy)phenyl substituted piperazine derivatives was synthesized (compounds **9a-m**, Scheme 3). Reductive amination of *m*-phenoxybenzaldehyde and 1-Boc-piperazine, followed by removal of the Boc with trifluoroacetic acid, gave compound **8**. Compound **8** was either acylated with EDCI activated carboxylic acid, or alkylated using reductive amination with substituted benzaldehyde and sodium triacetoxyborohydride, or sulfonylated with sulfonyl chloride in the presence of triethylamine to give compounds **9a-m** (Scheme 3). Several derivatives were found to possess anti-norovirus activity, however, potency and toxicity were highly sensitive to structural variations. The best compound in this group, tertiary sulfonamide **9l**, had a therapeutic index of 18.

In summary, this preliminary report demonstrates for the first time that functionalized piperazine compounds exhibit noteworthy anti-norovirus activity in a cell-based system. Two first-generation piperazine derivatives (compounds **6a** and **9l**) have been identified that could potentially serve as a starting point for further optimization studies in conjunction with mechanism of action studies aimed at identifying the molecular target(s) with which these compounds interact. Taken together, these results hold significant promise for the development of inhibitors directed against norovirus infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bergmann RJ, Gebhardt J, Gould E, Tucker P, Unge T, Hilgenfeld R, Neyts J. *Antiviral Research*. 2010; 87:162. [PubMed: 20471996]
2. Koo HL, Ajami N, Atmar RL, DuPont HL. *Discov Med*. 2010; 10:61. [PubMed: 20670600]
3. Atmar RL. *Food Environ Virol*. 2011; 2:117. [PubMed: 20814448]
4. Guiard J, Fiege B, Kitov PI, Peters T, Bundle DR. *Chemistry*. 2011; 17:7438. [PubMed: 21469230]
5. Rademacher C, Guiard J, Kitov PI, Fiege B, Dalton KP, Parra F, Buddle DR, Peters T. *Chemistry*. 2011; 17:7442. [PubMed: 21567493]
6. Rohayem J, Bergmann M, Gebhardt J, Gould E, Tucker P, Mattevi A, Unge T, Higenfeld R, Neyts J. *Antiviral Res*. 2010; 87:162. [PubMed: 20471996]
7. Dou D, Tiew KC, He G, Mandadapu SR, Aravapalli S, Alliston KR, Kim Y, Chang KO, Groutas WC. *Bioorg Med Chem*. 2011; 19:5975. [PubMed: 21925886]
8. Dou D, Mandadapu SR, Alliston KR, Kim Y, Chang KO, Groutas WC. *Bioorg Med Chem*. 2011; 19:5749. [PubMed: 21893416]
9. Dou D, Mandadapu SR, Alliston KR, Kim Y, Chang K-O, Groutas WC. *Eur J Med Chem*. 2011; 10.1016/j.ejmech.2011.10.019
10. DeSimone RW, Currie KS, Mitchell SA, Darrow JW, Pippin DA. *Comb Chem High Throughput Screen*. 2004; 7:473. [PubMed: 15320713]
11. Welsch ME, Snyder SA, Stockwell BR. *Curr Opin Chem Biol*. 2010; 14:347. [PubMed: 20303320]
12. Barelier S, Krimm I. *Curr Opin Chem Biol*. 2011; 15:469. [PubMed: 21411360]
13. Berkheij M, van der Sluis L, Sewing C, den Boer DJ, Terpstra JW, Hiemstra H, Iwema Bakker W, Ivan den Hoogenband A, van Maarseveen JH. *Tetrahedron Lett*. 2005; 46:2369.
14. Gillespie P, Goodnow RA. *Ann Rep Med Chem*. 2004; 39:293.
15. Wunberg T, Hendrix M, Hillisch A, Lobell M, Meier H, Schmeck C, Wild H, Hinzen B. *Drug Discov Today*. 2006; 11:175. [PubMed: 16533716]
16. All compounds were characterized by ^1H NMR and HRMS and had a >95% purity.
17. Chang KO, Sosnovtsev SV, Belliot G, King AD, Green KY. *Virology*. 2006; 2:463. [PubMed: 16843517]
18. Chang KO, George DW. *J Virol*. 2007; 22:12111. [PubMed: 17855555]
19. Chang KO. *J Virol*. 2009; 83:8587. [PubMed: 19515767]
20. Kim Y, Thapa M, Hua DH, Chang KO. *Antiviral Res*. 2011; 89:165. [PubMed: 21144866]
21. Kolb HC, Finn MG, Sharpless KB. *Angew Chem Int Ed*. 2001; 40:2004.
22. Moses JE, Moorhouse AD. *Chem Soc Rev*. 2007; 36:1249. [PubMed: 17619685]
23. Shen J, Woodward R, Kedenburg JP, Liu X, Chen M, Fang L, Sun D, Wang PG. *J Med Chem*. 2008; 51:7417. [PubMed: 19007204]
24. Mansour TS, Jin H. *Bioorg Med Chem Lett*. 1991; 1:757.

Figure 1.
General structure of piperazine derivatives.

Scheme 1.

Reagents and reaction conditions: i) R^1COOH or **2**/EDCI/DMF, then 1-benzyl piperazine dihydrobromide/TEA; ii) $R^2CH_2N_3$ /sodium ascorbate/ $CuSO_4$ /t-BuOH/ H_2O .

Scheme 2.

Reagents and reaction conditions: i) $\text{R}^3\text{CH}_2\text{NH}_2/\text{CH}_3\text{OH}/\text{r.t. to reflux}$; ii) 10% KOH/THF ; iii) $\text{EDCI}/\text{DMF}/1\text{-benzyl-piperazine dihydrobromide}/\text{TEA}$.

Scheme 3.

Reagents and reaction conditions: i) 1-Boc-piperazine/NaHB(OAc)₃/CH₂Cl₂; ii) TFA/CH₂Cl₂; iii) EDCI/R⁴COOH/DMF or R⁴CHO/NaHB(OAc)₃/CH₂Cl₂ or R⁴SO₂Cl/TEA/CH₂Cl₂.

Table 1

Compound	ED ₅₀ (μM) ^a	TD ₅₀ (μM)
1a	8.2	18.5
1b	2.8	11.4
1c	> 20	N/D ^b
1d	> 20	N/D
1e	> 20	N/D
1f	7.8	95.3
1g	> 20	N/D
1h	> 20	N/D
1i	> 20	N/D
3a	8.5	62.5
3b	>20	N/D
3c	>20	N/D
3d	>20	N/D
3e	>20	N/D
6a	7.2	155.4
6b	> 20	N/D
6c	> 20	N/D
6d	> 20	N/D
9a	3.8	23.5
9b	5.5	15.6
9c	> 20	N/D
9d	> 20	N/D
9f	> 20	N/D
9g	4.5	25.4
9h	> 20	N/D
9i	5.6	75.4
9j	> 20	N/D
9k	8.5	31.5
9l	6.7	121.2
9m	12.5	118.7

^a average of two independent experiments;

^b N/D: not done due to high ED₅₀ values.